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Biomedical Journal

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Original Article

Effect of Enzymatic pre-treatment of microalgae extracts on their anti-tumor activity

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ARTICLE INFO

Article history:
Received 22 January 2017
Accepted 24 October 2017
Available online 1 February 2018

Keywords:
Proliferation
Anti-cancer agents
Microalgae
Proteins
Enzymatic extraction

ABSTRACT

Background: There is an increasing need to find natural bioactive compounds for pharmaceutical applications, because they have less harmful side effects compared to their chemical alternatives. Microalgae (MA) have been identified as a promising source for these bioactive compounds, and this work aimed to evaluate the anti-proliferative effects of semi-purified protein extracted from MA against several tumor cell lines.

Methods: Tested samples comprised MA cell extracts treated with cellulase and lysozyme, prior to extraction. The effect of dialysis, required to remove unnecessary small molecules, was also tested. The anti-cancer efficacies of the dialyzed and undialyzed extracts were determined by measuring cell viability after treating four human cancer cell lines, specifically A549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), MDA MB-435 (human melanoma), and LNCap (human prostate cancer cells derived from a metastatic site in the lymph node). This was compared to the effects of the agents on the human BPH-1 cell line (benign human prostate epithelial cells). The t-test was used to statistically analyze the results and determine the significance.

Results: Against LNCap and A549 cells, the performance of cellulase-treated extracts was better (with p-values < 0.05, as compared to the control) than that of lysozyme-treated preparations (with p-values mainly > 0.05, as compared to the control); however, they had similar effects against the other two tumor cell lines (with p-values mainly < 0.05, as compared to the control). Moreover, based on their effect on BPH-1 cells, extracts from lysozyme-treated MA cells were determined to be safer against the benign prostate hyperplasia cells, BPH-1 (with p-values mainly > 0.05, as compared to the control). After dialysis, the performance of MA extracts from lysozyme-treated cells was enhanced significantly (with p-values dropping to < 0.05, as compared to the control).

Conclusions: The results of this work provide important information and could provide the foundation for further research to incorporate MA constituents into pharmaceutical anti-cancer therapeutic formulations.

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Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2017.10.003

At a glance commentary

Scientific background on the subject

Microalgae are promising sources of proteins that may have anti-tumor activity. Enzymes have been used to disrupt the rigid cell wall of microalgae for enhanced extraction of proteins, without denaturation. It was important to evaluate the effect of the enzymatic treatment on the anti-cancer bioactivity of the proteins extracts.

What this study adds to the field

The study shows that proteins extracted from enzymatic treated microalgae cells have a good anti-tumor activity. The activity of the semi-purified proteins extracts was shown to increase significantly by dialysis. The results could provide the foundation for further research to incorporate microalgae constituents into pharmaceutical anticancer therapeutic formulations.

Chemotherapy is the most effective method currently available for the treatment of cancer. However, drug resistance is common and associated several harmful side effects, which presents major obstacles for the effective treatment of cancer. Microalgae (MA) have been identified as a promising source of molecules for various pharmaceutical applications [1]. These cells mainly consist of proteins, carbohydrates, lipids, and pigments. Extracts from MA have been shown to inhibit the growth of cancer cells *in vitro and in vivo*, without affecting non-transformed cells [2]. Interest has mainly focused on phenolic compounds of MA, extracted using ethanol [3]. However, it is believed that MA proteins might also have significant bioactivities, especially those having properties not found in other natural sources [4,5]. For effective extraction from MA cells, the rigid cell wall must be disrupted, allowing the solvent (used for extraction) to reach and dissolve the proteins [6]. The disruption method must be effective in breaking up cell walls, but at the same time, it must protect the fragile proteins from denaturation. The effectiveness of using two enzymes, namely lysozyme and cellulase, for MA cell wall disruption and enhanced protein extraction was discussed in our previous paper [7]. Although we previously reported that lysozyme pretreatment was more effective in enhancing the extraction of proteins from MA, it is important to evaluate the effect of the treatment method on the anti-cancer bioactivity of the extracts, using various cancer and benign cell lines. For this, several cell lines were employed as models of the most common malignancies worldwide. A549 (a human epithelial lung carcinoma cell line), MCF-7 (human breast adenocarcinoma), MDA MB-435 (an M14 melanoma cell line) were used. In addition, LNCap and BPH-1 cells (prostate cancer epithelial cells and a benign prostate cell line, respectively) were also chosen for comparison. As mentioned earlier, the increasing demands for new therapeutic pharmaceutical drugs with low side effects have diverted the attention more then ever towards natural resources. Particularly, due to the diverse structural forms and biological activities of marine microalgae, their chemicals can be used as a valuable source of molecules for new drug development, including novel anti-cancer compounds [8]. The aim of this work was to identify the MA that potentially contains effective biochemical and chemical anti-cancer agents. Although using miscoalgae appears to be very promising, the rigid walls of microalgae cells need to be disrupted for efficient extraction of their bioactive compounds. Therefore, this work looks into assessing the effect of enzymatic disruption of cells walls, which was shown to be more advantageous than other conventional pre-treatment techniques, on the anti-tumor activity of the extracts.

Materials and methods

Enzymes, chemicals, and strains

Enzymes and other chemicals were purchased from Sigma–Aldrich Inc., USA. All MA strains used in this work were fresh water. *Chlorella* sp. was obtained from a local marine research center in Umm Al-Quwain, UAE. *Scenedesmus* sp. was kindly provided by Algal Oil Limited, Philippines. A mixed culture of MA was obtained from Ras Al-Khaimah Malaria Centre, UAE. This culture was isolated by serial dilutions followed by streaking on an agar medium, which was incubated until colonies appeared. An individual dominant colony was isolated and inoculated into sterilized Bold Basal medium (BBM), and this species was referred to as *M.C.* sp. in this work. The composition of the medium and the growth procedure are described in our previous paper [7].

Sample extraction and dialysis

The methods of MA extraction were detailed in our previous paper [7]. Briefly, samples were extracted from 1 g wet harvested MA cells, which was mixed with 3.25 mL of 1 mg/mL lytic enzyme solution (lysozyme or cellulase) and 7.5 mL of 0.1 M phosphate buffer solution (PBS) of pH 7.00 and pH 5.00 for lysozyme and cellulase pre-treatments, respectively. These conditions are the respective optimum conditions for each enzyme. Distilled water (9.25 mL) was added to bring the volume to 20 mL and the mixture was incubated in a water bath shaker (SCT-106.026, USA) at 37 °C and 100 rpm for 8 h. The cells were separated by centrifugation at 6000 rpm for 30 s and the supernatant was collected as the semi-purified protein sample. Total protein yields were determined as described in our previous publication [7]. A list of samples and their abbreviations used in this work are shown in Table 1.

The MA extract samples were dialyzed for 24 h against pure water in dialysis membrane tubes (100 Da cut-off), (100-500 Dalton molecular weight cut-off, MWCO), Spectrum™,

Table 1 Names of microalgae extracts from different strains treated with cellulase and lysozyme.

Microalgae species	Extracts obtained by cellulase treatments	Extracts obtained by lysozyme treatments
Chlorella sp.	C-Ch	L-Ch
M.C. sp.	C-Mc	L-Mc
Scenedesmus sp.	C-Sc	L-Sc